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Pharmacological targeting of neurotensin response by diet-derived EGCG in macrophage-differentiated HL-60 promyelocytic leukemia cells

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ABSTRACT

The neurotensin (NTS)/neurotensin receptor (NTSR) axis in chronic B cell leukemia regulates B cell proliferation. We questioned whether a similar NTS/NTSR axis was functional in acute myeloid leukemia (AML), and to what extent it could become a pharmacological target for epigallocatechin-3-gallate (EGCG), a diet-derived polyphenol with chemopreventive properties. Analysis of NTS, NTSR1, NTSR2, and NTSR3 gene expression in AML patients using Kaplan-Meier plots highlighted NTSR3 as a poor clinical prognosis biomarker. EGCG was tested on NTSR3 function and gene/protein expression in undifferentiated and phorbol 12-myristate 13-acetate (PMA)-differentiated HL-60 cells, an acute promyelocytic leukemia subset cell line model from AML, which can differentiate into $CD11b^{High}/CD14^{Low}$ adherent macrophages. We found that high level of gene, total protein, and cell surface expression of NTSR3 correlated with clinical samples of highly differentiated CD11b hematopoietic cells. Increased NTSR3 expression, NTS internalization, and matrix metalloproteinase-9 expression, was recapitulated in HL60-differentiated $CD11b^{High}$ macrophages, where chemotaxis in response to NTS was reduced through decreased Erk, MEK and Src signaling. EGCG prevented PMA-induced CD11b expression, NTS internalization and NTSR3-induced expression. Targeting of hematopoietic cell differentiation by EGCG could alter the NTS/NTSR3 signaling axis and possibly prevent the acquisition of an invasive phenotype.

1. Introduction

Soluble neuropeptide factors regulate a series of physiological functions in the central nervous system (CNS) and believed to stimulate the proliferation and migration of tumor cells [1]. As such, the oncogenic actions of neurotensin (NTS) have been described in different types of human cancers to primarily occur during the early stages of cell transformation [2]. Cell differentiation status may therefore play a crucial role in the extent of NTS physiological and pathological response levels. This has been reported, for instance, in the early stages of hemopoietic differentiation where NTS loses its physiological effect upon terminal differentiation, presumably through the down-regulation of NTS receptors (NTSRs) [3]. On the other hand, cell differentiation status is also shown to regulate NTSRs expression in undifferentiated primary cultured neurons as they remain undetectable in the undifferentiated state but do increase during differentiation [4]. Differential expression of NTS and NTSR must therefore be better understood in order to design optimized therapeutic interventions in line with specific cell differentiation status [5,6].

Levels of NTSR1 and NTSR2, two NTSRs characterized as G protein-coupled receptors, were recently assessed in normal and malignant human B lymphocytes, suggesting that a NTS/NTSR axis in chronic B cell leukemia may regulate the onset of B cell diseases [7,8]. The third NTS receptor NTSR3 is referred to as Sortilin and belongs to the Vps10p intracellular protein family, embedded in the endoplasmic reticulum/Golgi compartment [9,10]. NTSR3 is characterized by an intracellular cytoplasmic domain where sorting motifs regulate its subcellular distribution [11,12]. NTSR3 cellular functions include intracellular sorting of proteins, as well as engaging in signaling as a (co)receptor complexes [13]. Interestingly, when expressed alone, NTSR3 serves as a functional

Abbreviations: AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; BBB, blood-brain barrier; CNS, central nervous system; CSF, colony-stimulating factor; EGCG, epigallocatechin-3-gallate; EMT, epithelial-mesenchymal transition; MMP-9, matrix metalloproteinase-9; NTS, neurotensin; NTSR, neurotensin receptor; PMA, phorbol 12-myristate 13-acetate; TGF, transforming growth factor

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receptor regulating cell migration [14]. How its expression associates within a specific cell differentiation status remains unknown.

The human HL-60 promyelocytic leukemia cells are, among the several characterized leukemic cell lines, useful in understanding the process involved in cells which differentiate into mature myelomonocytic lineages [15]. As such, the tumor-promoting and protein kinase C activator phorbol 12-myristate 13-acetate (PMA) can elicite differentiation of HL-60 cells into mature functional monocytic/macrophage-like cells [16]. Macrophage differentiation further correlates with metastasis and inflammation events involving high secretion of collagenase MMP-9 [17–20]. Given chemokines play an important role in the recruitment of monocytes into cancer niches [21,22], and that upon chemotactic migration into the tumor niche, monocytes differentiate into macrophages [23], it was valuable for us to assess in this study the expression of the three NTSRs in HL-60 cells during their differentiation process into macrophages.

Transendothelial migration mechanisms of promyelocytic leukemia HL-60-derived macrophages has recently been inferred [24]. This was confirmed *in vitro* by the use of a blood-brain barrier (BBB) model and supports a potential CNS infiltration by circulating leukemic cells. However, the physiological function of the NTS/NTSR axis in macrophage chemotaxis and implication in neuro-inflammation in the CNS are poorly understood [25,26]. Exploiting its pharmacological properties and using diet-derived epigallocatechin-3-gallate (EGCG) may be envisioned in future anti-neuroinflammatory strategies. Accordingly, targeting of pro-inflammatory mediator signaling was suggested to prevent inflammation in neurodegenerative diseases [27,28]. Moreover, a protective effect of tea intake was recently reported among myeloid malignancies, which was more evident among acute myeloid leukemia (AML) [29]. Here, we specifically evaluated the NTS/NTSR signaling functions, and whether EGCG could prevent NTS-mediated chemotaxis upon macrophage differentiation.

2. Material and methods

2.1. Materials

Sodium dodecylsulfate (SDS), EGCG, PMA, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Canada (Oakville, ON). Electrophoresis reagents were from Bio-Rad (Mississauga, ON). The enhanced chemiluminescence (ECL) reagents were from Amersham Pharmacia Biotech (Baie d'Urfé, QC). Micro bicinchoninic acid protein assay reagents were from Pierce (Rockford, IL). The polyclonal antibodies against Src, phosphorylated Src, Akt, phosphorylated Akt, Erk, phosphorylated Erk, MEK, phosphorylated MEK were from Cell Signaling Technology Inc (Danvers, MA, USA), and NTSR3/Sortilin was from Abcam (Cambridge, UK). The monoclonal antibody against GAPDH was from Advanced Immunochemical Inc. (Long Beach, CA). Horseradish peroxidase-conjugated donkey anti-rabbit and anti-mouse IgG secondary antibodies were from Jackson ImmunoResearch Laboratories (West Grove, PA).

2.2. Kaplan-Meier survival plot and hierarchical differentiation trees analysis

A Kaplan-Meier survival plot analysis was performed using the Bloodspot (http://servers.binf.ku.dk/bloodspot/) algorithm, which provides a plot of gene expression in hematopoietic cells at different maturation stages based on curated microarray data [30]. For each of the four genes tested, we questioned the overall survival using the "Acute myeloid leukemia (AML) vs normal" dataset as follows: NTS (206291_at), NTSR1 (207360_s_at), NTSR2 (206899_at), and NTSR3 (SORT1, 212807_s_at). Human AML NK-AML and whole bone marrow cells [31] were compared to their nearest normal counterpart [32]. Browsing of human hematopoietic gene-expression fingerprints was also retrieved from that web-based database containing a large number of high-quality data set in hematopoietic cell types.

2.3. Cell culture

The human HL-60 acute promyelocytic leukemia cell line was purchased from American Type Culture Collection (Manassas, VA) and maintained in Iscove`s modified Dulbecco's medium (Gibco Invitrogen Cell Culture Systems, Burlington, ON) containing 20% (v/v) fetal bovine serum (FBS) (HyClone Laboratories, Logan, UT), 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and were cultured at 37 °C under a humidified atmosphere containing 5% CO_2. Currently, different experimental protocols define PMA-mediated HL-60 cells differentiation into "macrophage-like cells" in time, alone or in combination with other molecules. HL-60 macrophage differentiation conditions are herein defined as the adherent subpopulation of HL-60 cells immediately harvested upon 24 h treatment with 30 μM PMA.

2.4. Immunoblotting procedures

Undifferentiated suspensions or adherent PMA-differentiated HL-60 cells were lysed and proteins were separated by SDS–polyacrylamide gel electrophoresis (PAGE). After electrophoresis, proteins (30 μg) were electrotransferred to polyvinylidene difluoride membranes, which were then blocked for one hour at room temperature with 5% nonfat dry milk in Tris-buffered saline (150 mM NaCl, 20 mM Tris-HCl, pH 7.5) containing 0.3% Tween-20 (TBST; Bioshop, TWN510-500). Membranes were further washed in TBST and incubated with the respective anti-primary antibodies (1/1,000 dilution) in TBST containing 3% BSA and 0.1% sodium azide (Sigma-Aldrich Canada, S2002), followed by a 1 h incubation with horseradish peroxidase-conjugated donkey anti-rabbit or anti-mouse IgG at 1/2,500 dilutions in TBST containing 5% nonfat dry milk. Immunoreactive material was visualized by ECL.

2.5. Gelatin zymography

Gelatin zymography was used to assess the extent of proMMP-9 gelatinolytic activity in serum-starved undifferentiated vs PMA-treated HL-60 cells as previously described [18]. Briefly, an aliquot (20 μl) of the culture medium was subjected to SDS-PAGE in a gel containing 0.1 mg/ml gelatin, a substrate that is efficiently hydrolyzed by proMMP-9. The gels were then incubated in 2.5% Triton X-100 and rinsed in nanopure distilled H_2O. Gels were further incubated at 37°C for 20 h in 20 mM NaCl, 5 mM $CaCl_2$, 0.02% Brij-35, 50 mM Tris-HCl buffer, pH 7.6, then stained with 0.1% Coomassie Brilliant blue R-250 and destained in 10% acetic acid, 30% methanol in H_2O. Gelatinolytic activity was detected as unstained bands on a blue background.

2.6. Total RNA isolation, cDNA synthesis and real-time quantitative PCR

Total RNA was extracted from cell suspensions or monolayers using TriZol reagent (Life Technologies, Gaithersburg, MD). For cDNA synthesis, 2 μg of total RNA were reverse-transcribed using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). cDNA was stored at −80 °C prior to PCR. Gene expression was quantified by real-time quantitative PCR using iQ SYBR Green Supermix (Bio-Rad, Hercules, CA). DNA amplification was carried out using an Icycler iQ5 (Bio-Rad, Hercules, CA) and product detection was performed by measuring binding of the fluorescent dye SYBR Green I to double-stranded DNA. The QuantiTect primer sets were provided by Qiagen (Valencia, CA): MMP-9 (QT00040040), NTSR1 (Hs_NTSR1_1_SG, QT00018494), NTSR3/Sortilin (Hs_SORT1_1_SG, QT00073318), PPIA (Hs_PPIA_4_SG, QT01866137), GAPDH (Hs_GAPDH_1_SG, QT00079247), and β-Actin (Hs_Actb_2_SG, QT01680476). The primer sets for NTSR2 were provided by Bio-Rad (10041595). The relative quantities of target gene mRNA compared against two internal controls, GAPDH and β-Actin mRNA, were

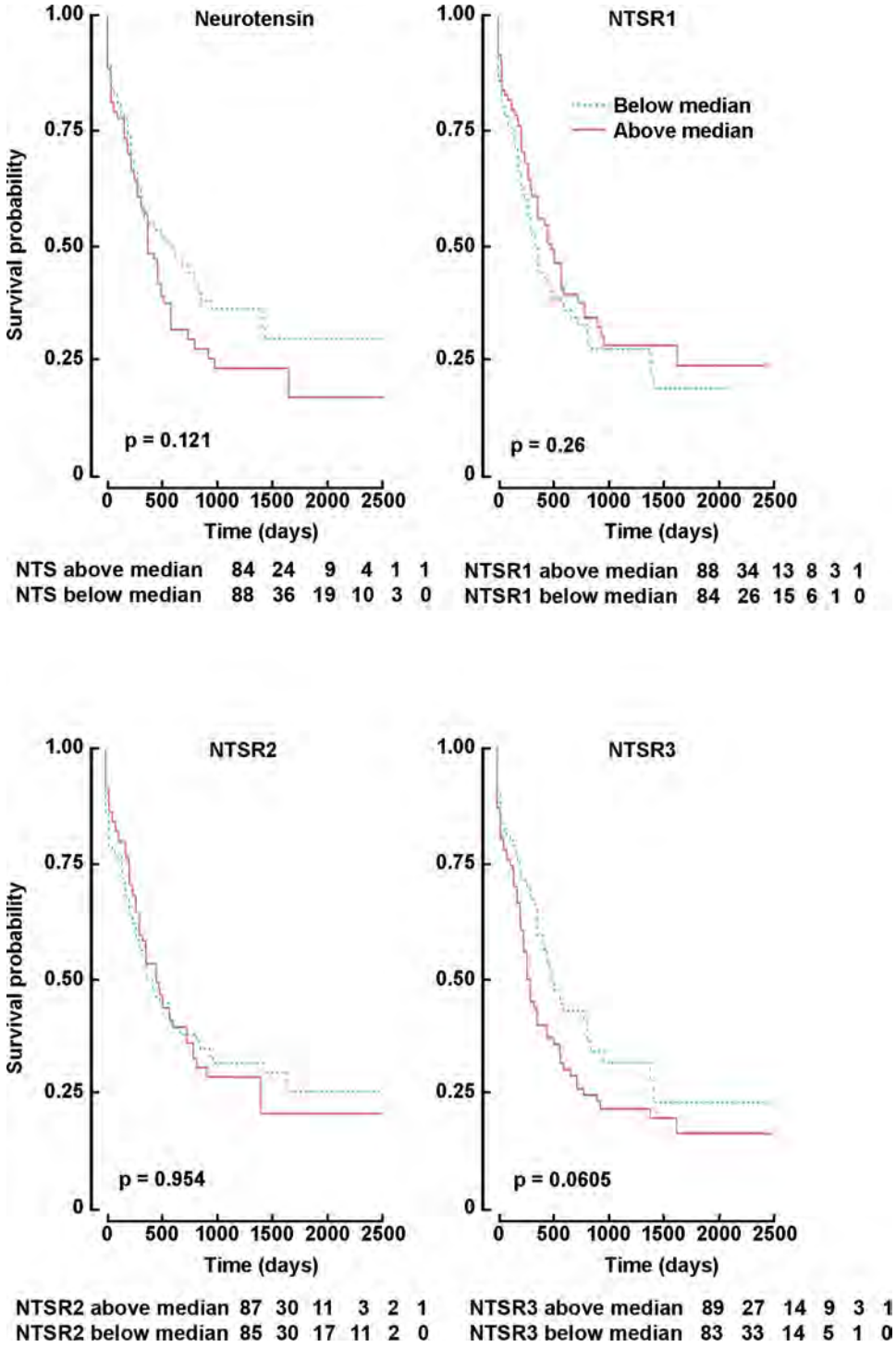

Fig. 1. High neurotensin and NTSR3 gene expression correlate with poor prognosis in AML patients. Kaplan-Meier analysis was performed using transcriptional programs (http://servers.binf.ku.dk/bloodspot/) addressing a database of gene expression profiles from healthy and malignant haematopoiesis [30]. The four panels show a survival plot based on a high-quality AML data set from The Cancer Genome Atlas (TCGA) displaying a full Kaplan-Meier analysis of Neurotensin, NTSR1, NTSR2, and NTSR3. The survival plots are only available for human data sets sharing probes with the microarray platform used by the TCGA. Blue lines show patients with gene expression below median levels, whereas red lines show patients with gene expression above median. Numbers at risk are shown for each gene tested in all panels.

measured by following a ΔCT method employing an amplification plot (fluorescence signal vs. cycle number). The difference (ΔCT) between the mean values in the triplicate samples of target gene and those of GAPDH and β-Actin mRNAs were calculated by iQ5 Optical System Software version 2.0 (Bio-Rad, Hercules, CA) and the relative quantified value (RQV) was expressed as 2-ΔCT.

2.7. Cell migration assay

Cell migration assay experiments were carried out using the Real-Time Cell Analyzer (RTCA) Dual-Plate (DP) Instrument of the xCELLigence system (Roche Diagnostics). Suspension cells or trypsinized adherent cells were seeded (20,000 cells/well) onto CIM-Plates 16 (Roche Diagnostics). These migration plates are similar to conventional Transwells (8 μm pore size) but with gold electrode arrays on the bottom side of the membrane to provide a real-time measurement of cell migration. Prior to cell seeding, the underside of the wells from the upper chamber was coated with 25 μL of 0.15% gelatin in PBS and incubated for 1 h at 37 °C. Cell migration was monitored for 6 h in the exclusive response or not to 10 μM NTS. The impedance values were measured by the RTCA DP Instrument software and were expressed in arbitrary units as Normalized Cell Migration Index. Each experiment was performed three times in triplicate.

A.

Abbreviation	Name	Immunophenotype
HSC	Hematopoietic stem cell	Lin- CD34+ CD38- CD90+ CD45RA-
MPP	Multipotential progenitors	Lin- CD34+ CD38- CD90- 45RA-
CMP	Common myeloid progenitor cell	Lin- CD34+ CD38+ CD45RA- CD123+
GMP	Granulocyte monocyte progenitors	Lin- CD34+ CD38+ CD45RA+ CD123+
MEP	Megakaryocyte-erythroid progenitor cell	Lin- CD34+ CD38+ CD45RA- CD123-
early_PM	Early Promyelocyte	Lin- FSChi SSCint CD34- CD15int CD49dhi CD33hi CD11b- CD16-
late_PM	Late Promyelocyte	Lin- FSChi SSChi CD34- CD15hi CD49dhi CD33hi CD11b- CD16-
MY	Myelocyte	Lin- FSChi SSChi CD34- CD15hi CD49dhi CD33hi CD11bhi CD16-
MM	Metamyelocytes	Lin- FSChi SSChi CD34- CD15hi CD49d- CD33- CD11bhi CD16-
BC	Band cell	Lin- FSChi SSChi CD34- CD15hi CD49d- CD33- CD11bhi CD16int
PMN	Polymorphonuclear cells	Lin- FSChi SSChi CD34- CD15hi CD49d- CD33- CD11bhi CD16hi
Mono	Monocytes	CD14+ CD16-

B.

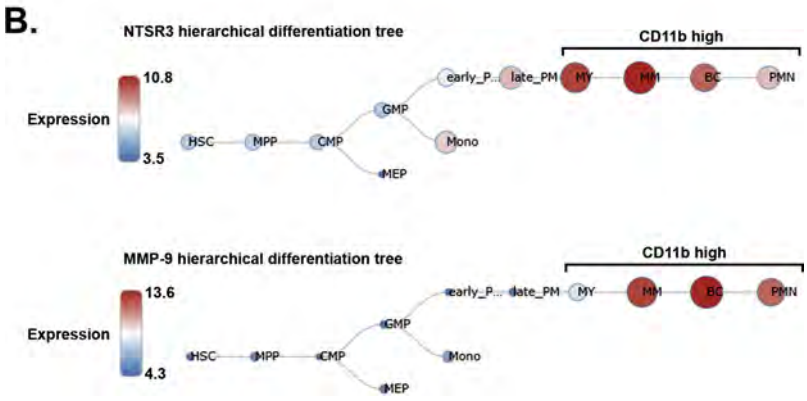

Fig. 2. Hierarchichal differentiation tree shows increased NTSR3 and MMP-9 expression in high CD11b expressing cells. An interactive hierarchical differentiation tree was generated using the "Bloodspot" interface. A) Immunophenotyping details are shown for each of the cells' characteristics. B) NTSR3 and MMP-9 expression intensity at each of the differentiated status is expressed, with the highest expression observed for each gene in high CD11b expressing cells. The colour in the nodes represents the median expression of the queried gene. To accentuate the display in the trees, node size is also proportional to gene expression.

2.8. Flow cytometry staining protocol and data acquisition

Following the *in vitro* PMA-mediated stimulation of HL-60 cells, culture cells in suspension and cells detached from adhesion were analyzed by multicolor flow cytometry. An Fc receptor-blocking reagent (Miltenyi Biotec) was used to prevent non-specific binding of antibodies. Subsequently, cells were stained and incubated for 1 h at 4 °C with a cocktail of specific antibodies. To exclude dead cells, a viability dye kit (Invitrogen) was used. Cells were then fixed with a fixation buffer (BD Cytofix) and acquired on a BD Fortessa-X20 (BD Biosciences) flow cytometer. FlowJo V10.2 (FlowJo LLC) software was used to assess the frequencies and mean fluorescence intensities of various subsets of cells. Monocytes and macrophages were identified by their characteristic forward and side light scatter properties. Cells of the hematopoietic lineage were identified through their expression of CD45 (anti-CD45 Alexa Fluor 700 Clone HI30, BD Biosciences). Expressions of CD14 (BV786 mouse anti-human CD14 Clone M5E2, BD Biosciences) were used to identify classical, intermediate and non-classical monocytes. CD11b (APC mouse anti-human CD11b Clone D12, BD Biosciences) was used to identify cells known to exhibit phagocytosis, chemotaxis and cellular activation. Expression of NTSR3/Sortilin was assessed with the human Sortilin Alexa Fluor® 488-conjugated antibody (Monoclonal mouse IgG1 Clone 334703, R&D System).

2.9. Statistical data analysis

Unless otherwise stated, data are representative of three or more independent experiments. Statistical significance was assessed using Student's unpaired t-test. Probability values of less than 0.05 were considered significant and an asterisk identifies such significance in the figures. Error bars in all figures represent standard error means (s.e.m.) values.

3. Results

3.1. High neurotensin and NTSR3 gene expression correlate with poor prognosis in AML patients

Expression of neurotensin (NTS) and of its receptors (NTSR) in T lymphocytes [33], macrophages [34], dendritic cells [35], and during B lymphocytes prosurvival signaling [8] suggests that NTS/NTSR axis could modulate immune response and become an appealing pharmacologic target of therapeutic interest. Modulations in the NTS/NTSR axis therefore warrants more investigation in blood malignancy. We thus addressed the levels of NTS, NTSR1, NTSR2, and NTSR3 transcript expression in AML patients using Kaplan-Meyer analysis as described in the Methods section (Fig. 1). Although limited in clinical samples, data showed no significance for NTSR1 and NTSR2 in the same AML patients where a potential correlation between poor prognosis and high levels of both NTS and NTSR3 expression was observed. This analysis emphasizes on NTSR3 expression as a potential poor prognosis biomarker in AML disease progression, and warrants further investigation regarding its molecular regulation. When exploring whether a particular karyotype or mutational status occurred in patients with high NTSR3 expression, significant enrichment was found in AML patients with $p < 0.05$ for inv(16) and trisomy 13, with $p < 0.01$ for t(8, 16), t(8,21), and t(11q23)/MLL, and with $p < 0.001$ for t(15,17).

3.2. Hierarchical differentiation tree shows increased NTSR3 and MMP-9 expression in CD11b^{high} expressing cells

Interactive hierarchical differentiation trees were generated using the Bloodspot algorithm as described in the Methods section. Immunophenotyping details were first described and used to discriminate between the several cells statuses involved during hematopoietic differentiation (Fig. 2A). Interestingly, NTS3 expression intensity was found to increase as the cells differentiated towards a high CD11b expression status (Fig. 2B, upper tree). More importantly, we also observed that matrix metalloproteinase (MMP)-9 gene expression also correlated with similar differentiated statuses (Fig. 2B, lower tree). MMP-9 is well documented to be involved in the disruption of the BBB [20,36] and in the setting of inflammatory processes [37]. The color in the nodes represents the median expression of the queried gene. To accentuate the display in the trees, node sizes are also proportional to gene expression.

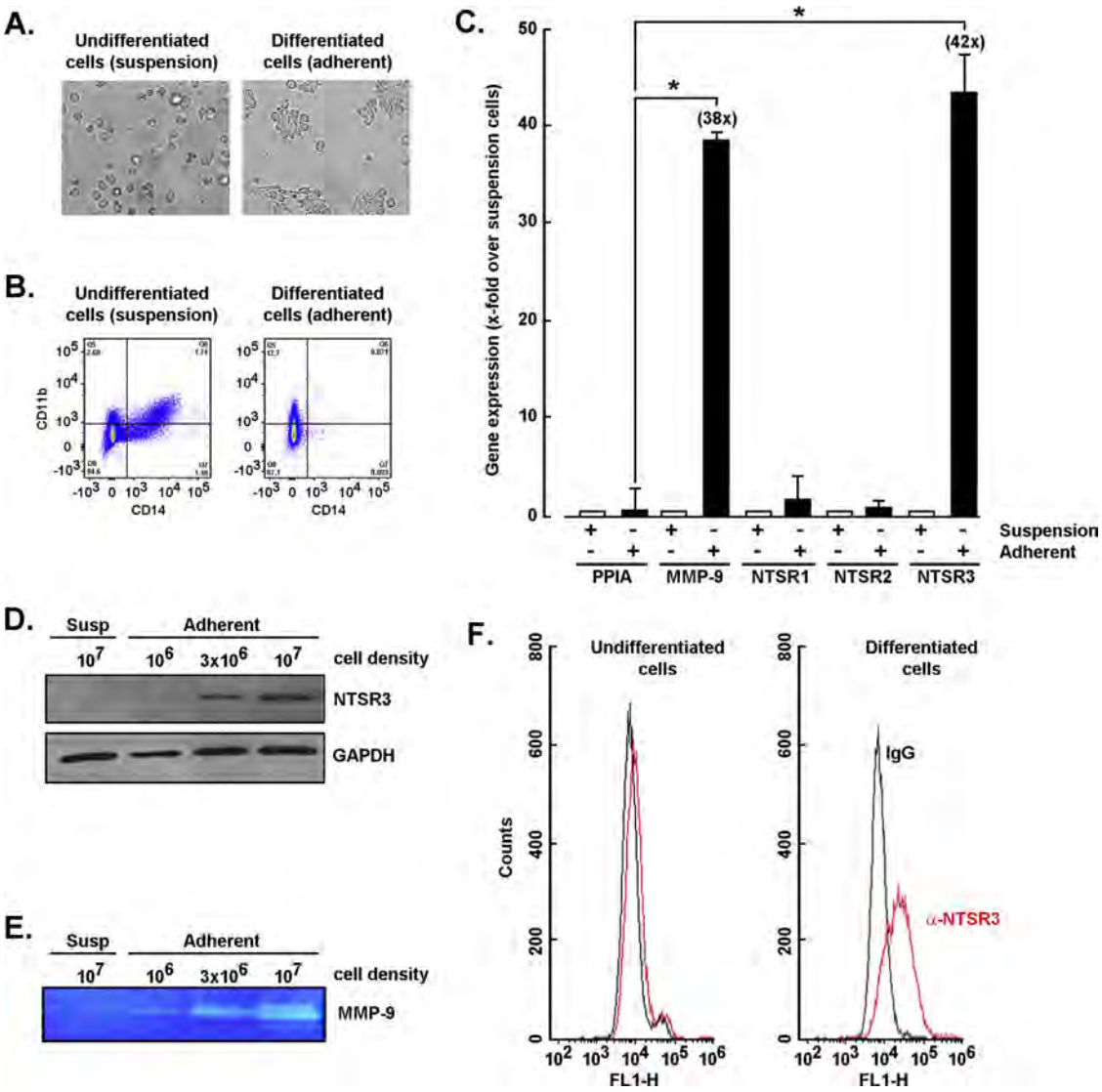

Fig. 3. Increased NTSR3 and MMP-9 expression upon PMA-induced differentiation of promyelomonocytic HL-60 suspension cells into macrophage-like adherent cells. A) Undifferentiated HL-60 cells classically grow in suspension and can be differentiated into adherent macrophage-like cells with 0.3 μM PMA. B) CD11b and CD14 cell surface immunophenotyping was performed by flow cytometry as described in the Methods section to asses the extent of macrophage differentiation. Serum-starved undifferentiated HL-60 cell cells were cultured and differentiated or not with 0.3 μM PMA for 24 h. C) Cells were harvested in order to isolate total RNA and gene expression assessed by RT-qPCR as described in the Methods section. D) Lysates were also analysed for protein content by Western blotting and immunodetection performed as described in the Methods section in order to detect NTSR3 and GAPDH. E) The conditioned media of the above experimental conditions were harvested and the extent of proMMP-9 secretion assessed using gelatin zymography. F) Cell surface immunophenotyping of NTSR3 was performed in undifferentiated HL-60 cells as well as in PMA-mediated differentiated macrophage-like cells as described in the Methods section. Probability values of less than 0.05 were considered significant and an asterisk identifies such significance.

3.3. PMA-induced differentiation of promyelocytic HL-60 suspension cells into macrophages triggers NTSR3 and MMP-9 expression

In order to address how any modulation of the NTS/NTSR axis correlates with the level of mobilization of the cells, we chose to work with the HL-60 acute promyelocytic leukemia cell line model, which was appropriately reclassified as an acute myeloblastic leukemia with maturation cell model FAB-M2, therefore better approximating AML [38–40]. Such model allows one to further assess validated attractive molecular processes involved *in vitro* differentiation into granulocytic/monocytic/macrophage lineage [41]. Of particular interest, PMA-mediated differentiation of HL-60 cell suspensions triggered intense adherence to plastic (Fig. 3A), and expression of a $CD14^{low}/CD11b^{high}$ macrophage-like phenotype (Fig. 3B). When gene expression was compared between adherent differentiated cells and undifferentiated cells in suspension, NTSR3 was significantly induced as well as MMP-9 (Fig. 3C). NTSR3 increased expression in differentiated adherent cells was further confirmed at the protein level in cell lysates (Fig. 3D), whereas secreted MMP-9 was also found to be increased as assessed by zymography from isolated conditioned culture media (Fig. 3E). NTSR3 cell surface immunophenotyping was performed by flow cytometry, and NTSR3 levels confirmed to increase upon cell differentiation (Fig. 3F).

3.4. Neurotensin internalization increases in PMA-differentiated macrophage-like HL-60 cells and requires functional NTSR3

We next assessed the functionality of NTSR3 as it is, in contrast to NTSR1 and NTSR2 which rather transduce intracellular signaling upon ligand binding, involved in NTS trafficking from the cell surface within subcellular compartments [42]. Undifferentiated and PMA-differentiated cells were incubated with fluorescent NTS, and levels of

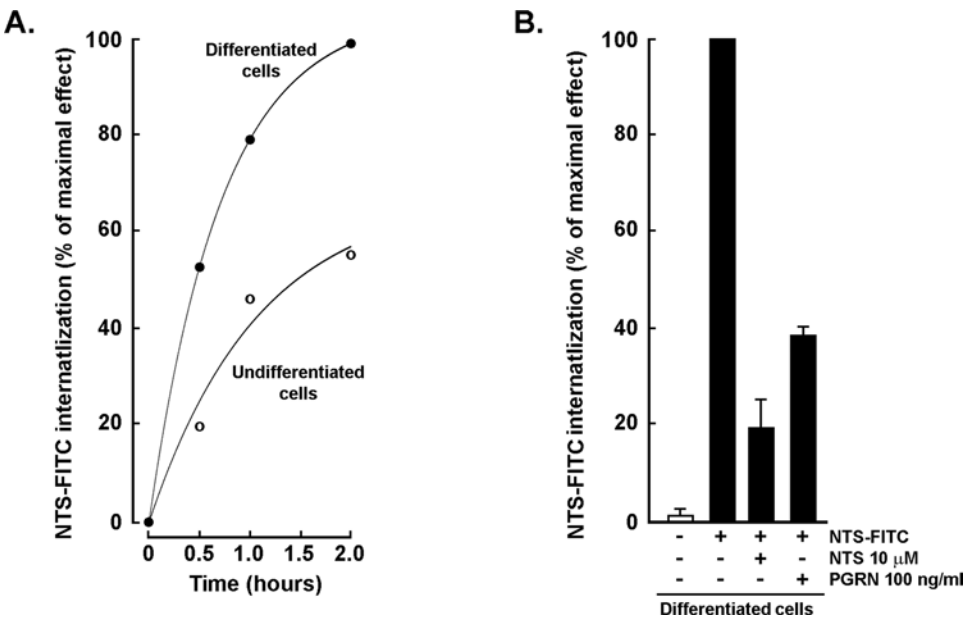

Fig. 4. Neurotensin internalization is increased in PMA-differentiated HL-60 macrophages and requires NTSR3. A) A representative uptake assay of fluorescent NTS-FITC was performed as described in the Methods section in undifferentiated HL-60 cell suspensions (open circles) and in 0.3 μM PMA-differentiated macrophage-like cells (closed circles). NTS-FITC internalization for up to 2 h was measured using flow cytometry. B) NTS-FITC internalization was measured in PMA-differentiated cells in the presence or not of non-fluorescent excess NTS (10 μM), or progranulin (PGRN, 100 ng/ml). Extent of fluorescence was measured using flow cytometry.

intracellular fluorescence assessed by flow cytometry. We found that NTS internalization was significantly increased in adherent macrophage-like cells (Fig. 4A), and that excess of unlabeled NTS or NTSR3 ligand progranulin efficiently competed with fluorescent NTS uptake validating NTSR3 cell surface involvement (Fig. 4B).

3.5. Differential chemotactic response to neurotensin between undifferentiated and differentiated macrophage-like HL-60 cells

In order to address the level of cell mobilization upon differentiation, we next performed a real-time cell migration assay and observed that macrophage-like adherent HL-60 cells had significant higher migratory potential than their undifferentiated counterparts (Fig. 5A). When chemotactic response to NTS was assessed, cell migration was found to increase dose-dependently in response to NTS in undifferentiated cells (Fig. 5B), whereas NTS rather triggered a decrease in differentiated macrophage-like cell migration (Fig. 5C). In order to document some of the downstream signaling intermediates involved in the NTS/NTSR axis, cell lysates from undifferentiated and differentiated macrophage-like HL-60 cells were harvested upon NTS stimulation and Western blotting performed. We observed that NTS triggered phosphorylation of the mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway (Fig. 5D, first two columns). Increased basal migration of the macrophage-like differentiated HL-60 cell migration was also found to correlate with increased levels of phosphorylated MEK/Erk as well as of phosphorylated Src (Fig. 5D, third column). Interestingly, NTS treatment of these cells resulted in the decrease of the phosphorylated status of MEK/Erk/Src (Fig. 5D, last column), in line with the decreased cell migration responsiveness to NTS (Fig. 5C).

3.6. EGCG prevents PMA-induced NTSR3 gene expression in differentiated macrophages

Green tea-derived polyphenol epigallocatechin-3-gallate (EGCG) was recently found to alter several processes upon PMA-induced macrophage differentiation of HL-60 cells, and included mRNA stabilizing factor HuR-mediated transcriptional control of MMP-9 expression [17], efficient targeting of NF-κB downstream gene products [28], as well as targeting platelet-derived growth factors-mediated macrophage chemotaxis [43]. We therefore assessed whether EGCG further altered NTSR3 expression and any aspects associated with differentiation into macrophages. Cell surface immunophenotyping was performed on undifferentiated HL-60 cells and compared to that of PMA-differentiated macrophage cells in the presence or not of EGCG. We found that EGCG did not prevent cells from adhering to culture plates. However, whereas the expression of CD14 remained low, that of CD11b was reduced to levels comparable to undifferentiated cells (Fig. 6A). Furthermore, EGCG prevented the increases in NTSR3 cell surface (Fig. 6B) and transcript (Fig. 6C) levels.

3.7. EGCG potentiates the reduced chemotactic response of HL-60 differentiated macrophages

Whether EGCG's ability to decrease NTSR3 expression affected NTS chemotactic response was next assessed. We found that EGCG reduced basal migration of undifferentiated cells by ~20%, while concomitantly prevented NTS-induced chemotaxis (Fig. 7A). On the other hand, reduced chemotactic response was observed in response to NTS in macrophage-like HL-60 cells and EGCG further potentiated that effect (Fig. 7B).

4. Discussion

Regulations of immune and inflammatory processes are believed to, in part, take place upon NTS/NTSR signaling axis in T lymphocytes, macrophages and dendritic cells. Whereas NTS was reported to regulate mast cell activation, neutrophil chemotaxis, NO generation and cytokine production, its co-regulation with NTSRs remains unclear in several types of cancers. For instance, gene expression was studied in two malignant B cell diseases and showed that NTSR2 was increased, NTSR1 was decreased, and NTS was unexpressed in B cell leukemia patient's cells [8]. However, these expressions did not significantly change in large diffuse B cell lymphoma lymph nodes as compared with benign ones. Recently, NTSR2 was suggested to be an essential driver of apoptosis resistance in B-cell chronic lymphocytic leukemia (B-CLL) cells, where it was highly expressed, in contrast to NTS levels, which were minimal in both B-CLL cells and patient plasma [7]. In the current study, we found that increased NTS and NTSR3 expression tended to correlate with poor prognosis in AML patients (Fig. 1). Overall and given the roles NTSRs play in cancer cell survival, targeting of the NTS/NTSR functions and signaling axis may legitimately represent a promising therapeutic target.

An arrest in the terminal differentiation of promyelocytes into

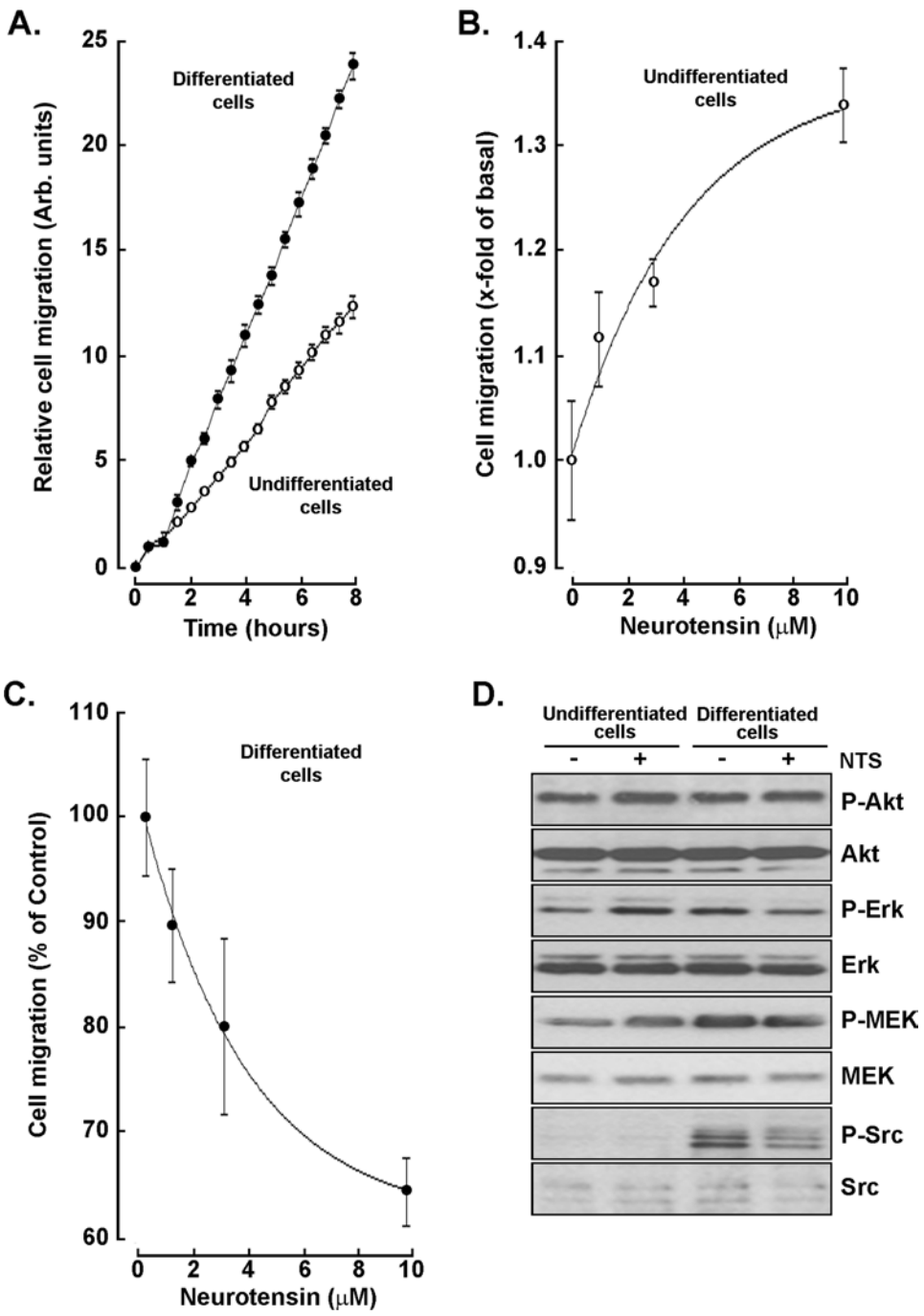

Fig. 5. Differential chemotactic response to neurotensin between undifferentiated and differentiated macrophage-like HL-60 cells. A) Real-time cell migration was performed using the xCELLigence system as described in the Methods section in undifferentiated HL-60 cell suspensions (open circles) and in 0.3 μM PMA-differentiated macrophage-like cells (closed circles). Chemotactic response to 10 μM NTS was performed and cell migration assessed in B) undifferentiated cells, and in C) 30 μM PMA-differentiated macrophage-like cells. D) Cell lysates were harvested upon 18 h NTS stimulation. Protein lysates were then processed for SDS-PAGE and Western blotting in order to assess the phosphorylation status of Akt, Erk, MEK, and Src as described in the Methods section.

granulocytes characterizes acute promyelocytic leukemia (APL) and represents 5–15% of all cases of AML [44]. In the current study, we specifically investigated the differential expression of NTSR1, NTSR2, and NTSR3 that occurs upon PMA-mediated differentiation into a macrophage-like phenotype using an HL-60 promyelocytic cell model. As such, HL-60 cells have more appropriately been classified as an acute myeloblastic leukemia with maturation cell model FAB-M2, therefore better approximating AML [38]. Among the three NTSRs tested, only the expression of NTSR3 was significantly triggered upon macrophage differentiation (Fig. 3), which supports the predicted poor patients' prognosis observed in Fig. 1. Furthermore, macrophage differentiation also correlated with increased chemotaxis, in part explained by the high MMP-9 levels. Recent evidence in pancreatic beta-TC3 cells suggests that NTSR2 and NTSR3 are crucial for the anti-apoptotic effect of NTS [45]. While NTSR1 was suggested as a prognosis marker in breast, lung, and head and neck squamous carcinomas [46], neither NTSR1 nor NTSR2 were here found differentially expressed between healthy and diseased leukemic patients, prompting to conclude NTSR3 as the best potent biomarker to further be investigated.

In this study, NTS/NTSR3 axis therefore appears to fulfill a significant role in the PMA-mediated differentiation process of promyelocytic HL-60 cells into a macrophage-like phenotype as this correlated with increases in both NTS internalization and cell migration. Furthermore, NTS internalization and NTSR3-induced expression in macrophages was found reversed upon treatment with chemopreventive diet-derived EGCG. Altogether, our observations add up and support those observed previously in HL-60 macrophage-like cells, and which documented the pleiotropic EGCG targeting including the MMP-9 mRNA stabilizing factor HuR [17], numerous NF-κB downstream gene products [18], as well as sphingosine-1-phosphate-mediated signaling

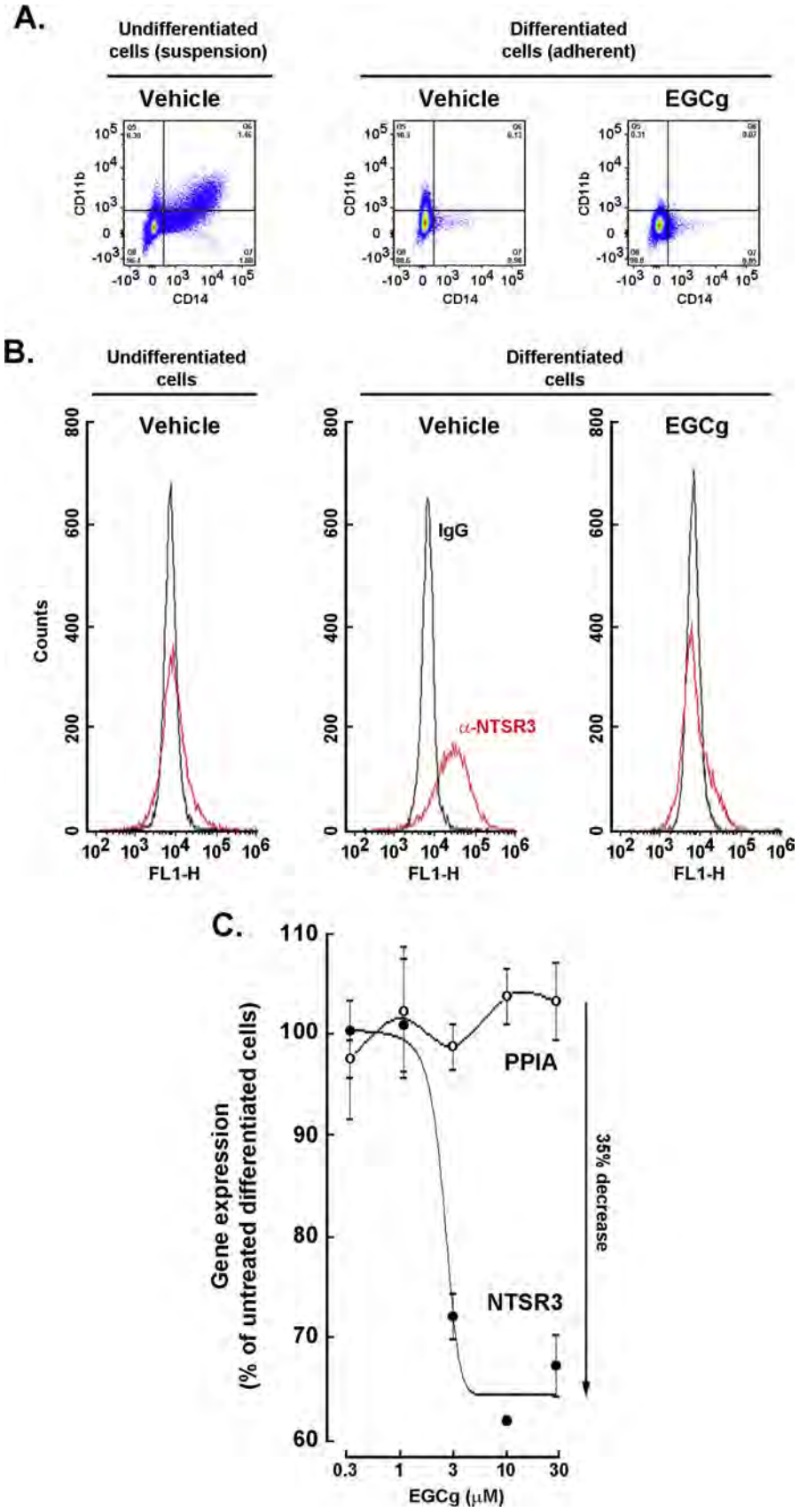

Fig. 6. EGCG prevents PMA-induced NTSR3 gene expression in differentiated macrophages. A) Undifferentiated HL-60 cell suspensions were differentiated into adherent macrophage-like cells with 0.3 μM PMA or a combination of PMA and 30 μM EGCG for 18 h in serum-deprived media. Cells were then immunophenotyped for CD11b and CD14 cell surface expression by flow cytometry as described in the Methods section. B) NTSR3 immunophenotyping was similarly performed as above on undifferentiated cells, and differentiated cells in the presence or not of 30 μM EGCG. C) Total RNA was isolated as described in the Methods differentiated cells in the presence or not of 30 μM EGCG and RT-qPCR performed in order to assess PPIA and NTSR3 gene expression levels.

[43]. EGCG targeting of NTSR3 cell surface expression and cellular function now demonstrates that this can possibly also alter several other NTSR3 functions such as those involved in the control of inflammation through regulation of cytokine secretion [47]. Recent evidence supporting such impact of EGCG on cytokine secretion was also provided on circulating mesenchymal stromal cells, which are

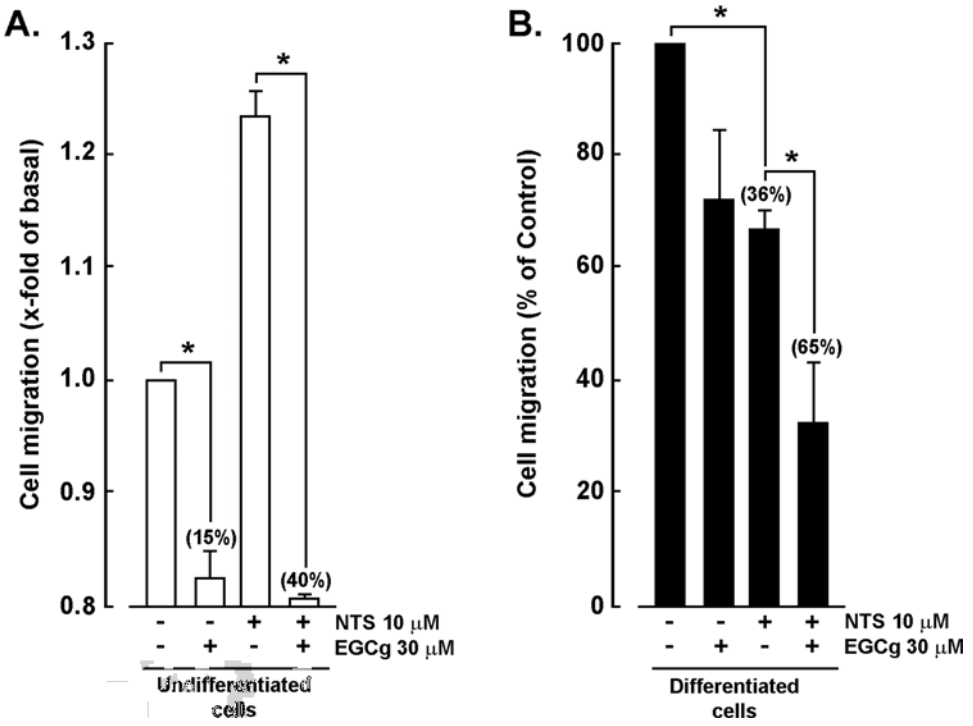

Fig. 7. EGCG potentiates the reduction in chemotactic response of HL-60 differentiated macrophages. A) Real-time cell migration assay was performed using the xCELLigence system as described in the Methods section. Chemotactic response to NTS in the presence or not of EGCG was performed in A) undifferentiated HL-60 cell suspensions, and in B) 0.3 μM PMA-differentiated macrophage-like cells. Probability values of less than 0.05 were considered significant and an asterisk identifies such significance. % decreases are shown in between brackets.

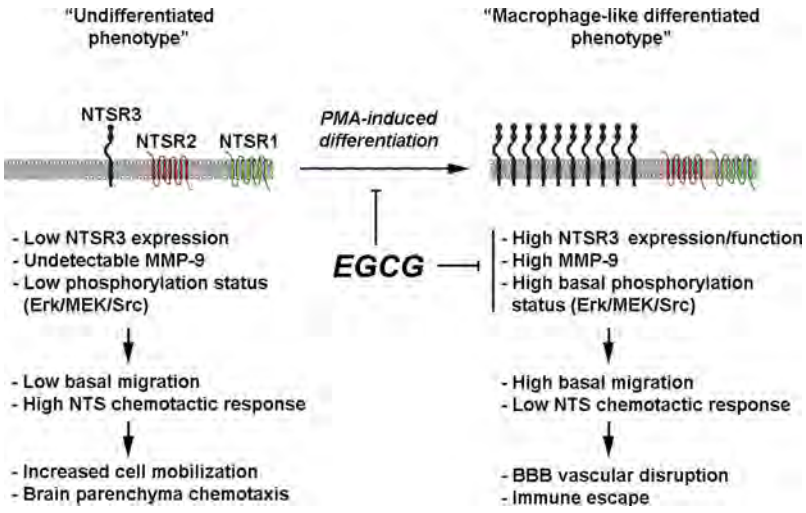

Fig. 8. Schematic representation of the molecular signature associated to the PMA-induced macrophage-like phenotype, and implications in brain immune evasion strategies. Undifferentiated promyelocytic HL-60 cells express basal levels of NTSR1, NTSR2, and NTSR3. PMA-induced differentiation triggers a macrophage-like phenotype associated with increased NTSR3 expression and function (high NTS internalization), as well as increased MMP-9 and phosphorylation status of Erk/MEK/Src. High basal migration is associated with lower NTS responsive index. Treatment with EGCG inhibits the acquisition of a macrophage-like phenotype, lowers NTSR3 and MMP-9 expression, and potentiates the NTS decrease in cell migration. Altogether, given immune cells can migrate into the brain parenchyma, acquisition of a macrophage-like phenotype may contribute to immune evasion strategies.

believed to be avidly recruited within vascularizing tumors [48,49], possibly through secretion of colony-stimulating factor (CSF)-2 and CSF-3 [50].

NTS implication in tumor growth processes includes anti-apoptotic and cell proliferation functions as previously reported in various types of human colon, prostate, lung, pancreas, and breast cancer cell lines [51]. Involvement of NTS was also reported in inflammation [52,53], cytokine production [54,55], enhancement of phagocytosis [56], and nitric oxide (NO) generation [57]. Tumor growth is associated with chronic inflammation and is mediated through various immune cells among which the highly infiltrative phenotype of macrophages account for ~30%-50% of the tumor mass [56]. NTS signaling has also recently found to cross-link inflammation and epithelial-mesenchymal transition (EMT)-related tumor migration [1]. NTS/NTSR signaling axis therefore plays an important role in the acquisition of an invasive phenotype which is in fact prerequisite to metastatic spread. How NTSR3 can participate to such process needs to be further investigated. Interestingly, NTSR3 association with transforming growth factor (TGF)-β family proteins was recently found to negatively regulate TGF-β signaling by enhancing lysosome-mediated degradation [58]. Critical roles have been inferred to TGF-β as a pro-tumorigenic factor regulating EMT induced by TGF-β [59]. Thus, targeting TGF-β signaling, especially at early stages of tumorigenesis, may be useful for the treatment or prevention of some cancers [60]. In light of this, the chemopreventive properties of EGCG have been reported to inhibit TGF-β-induced EMT [61–64] and cell invasion [65].

An important original and intriguing aspect of our study relies upon the dual functions that NTS appears to exert on cell migration upon HL-60 cell differentiation into macrophages. Whereas, NTS-mediated chemoattraction of undifferentiated promyelocytic HL-60 cells triggered Erk and MEK phosphorylation, it rather reduced migration once cells adopted a macrophage-like phenotype (Fig. 5D). While NTSR3 involvement in brain macrophage microglia migration was previously reported [66,67], here we show that increased NTSR3 cell surface expression correlated with increased migratory phenotype in differentiated macrophages through, in part, increased basal levels of

phosphorylated Erk/MEK and Src (Fig. 5D). This is in line with increased Src kinase activity which was recently shown to be associated with differentiating macrophages [68]. Additionally, NTSR3 knockdown led to dramatic decreases in Src phosphorylation and neuroendocrine tumor cells migration [69], as well as in triple-negative MDA-MB-231 cells [14]. In our study, NTS in conditions of high NTSR3 expression as found in macrophages, rather led to a decrease in Erk/MEK/Src phosphorylation status possibly because NTS was not made available for NTSRs signaling. A combination of two lines of evidence can, in part, explain such effect. The first evidence relies on the regulation of the subcellular localization of NTSR3 which controls NTS internalization and trafficking [70]. In fact, it is postulated that two distinct NTSR3 pools are responsible for either NTS endocytosis from the cell surface, or sorting of internalized NTS to the Trans-Golgi network. Regulation of the partitioning processes of these NTSR3 pools upon macrophage differentiation remains to be addressed and may favor NTS internalization and degradation. The second evidence relies on the cell surface heterodimerization capacity of NTSRs/NTSR3 complexes which can ultimately modulate NTS signaling [71]. As such, it was elegantly demonstrated that increased expression of NTSR3 led to increased NTSR1/NTSR3 complexes and decreased potency of NTSR1 to activate phospholipase C and to stimulate PI production [71]. Moreover, similarly to the effects observed in our study, the presence of increased NTSR3 lowered the sensitivity of NTS to stimulate the phosphorylation of MAP kinases [71]. Of interest, PMA-mediated shedding of a soluble form of NTSR3 was reported from HT29 colorectal cancer cell surface, where it was hypothesized to modulate NTS biological effects [72].

5. Conclusion

In conclusion, whereas we do acknowledge that further cell models must be tested including the use of CD34+ cells, our current study is the first that delineates a predicted correlation between NTS/NTSR3 biomarkers to poor clinical prognosis in AML patients. Next, we defined pleiotropic NTSR3 molecular functions associated with increased basal migratory phenotype upon macrophage differentiation, which will require to be further documented within other human AML cell lines and, more importantly, in AML cells derived from the bone marrow of AML patients. Given immune cells can migrate into the brain parenchyma by chemotaxis [73], addition of the increased MMP-9 involvement in vascular permeabilization, may further contribute to immune escape and pro-inflammatory processes associated with tumor development [74]. Our data support furthermore the MMP-9 functional regulation of invasive capacity in the occurrence of extra-medullar infiltrates of leukemia blast cells that occurs frequently during the onset of monocyte-related AML sub-types [75]. Finally, we provide further support for the chemopreventive properties of diet-derived EGCG (summarized in Fig. 8) through regulation of NTSR3 and MMP-9 expression at their mRNA level that may involve targeting of several upstream signaling pathways including Erk/MEK/Src and NF-κB targeting.

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Transparency statement

The lead author (the manuscript's guarantor) affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Declaration of Competing Interest

The authors declare that they have no conflict of interest with the contents of this article.

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References

[1] Y. Ye, P. Liu, Y. Wang, H. Li, F. Wei, Y. Cheng, L. Han, J. Yu, Neurotensin, a novel messenger to cross-link inflammation and tumor invasion via epithelial-mesenchymal transition pathway, Int. Rev. Immunol. 35 (2016) 340–350, https://doi.org/10.3109/08830185.2014.952412.
[2] Q. Ouyang, J. Zhou, W. Yang, H. Cui, M. Xu, L. Yi, Oncogenic role of neurotensin and neurotensin receptors in various cancers, Clin. Exp. Pharmacol. Physiol. 44 (2017) 841–846, https://doi.org/10.1111/1440-1681.12787.
[3] S.Y. Choi, H.D. Chae, T.J. Park, H. Ha, K.T. Kim, Characterization of high affinity neurotensin receptor NTR1 in HL-60 cells and its down regulation during granulocytic differentiation, Br. J. Pharmacol. 126 (1999) 1050–1056, https://doi.org/10.1038/sj.bjp.0702378.
[4] J. Chabry, F. Checler, J.P. Vincent, J. Mazella, Colocalization of neurotensin receptors and of the neurotensin-degrading enzyme endopeptidase 24-16 in primary cultures of neurons, J. Neurosci. 10 (1990) 3916–3921, https://doi.org/10.1523/JNEUROSCI.10-12-03916.1990.
[5] T.W. Moody, I. Ramos-Alvarez, R.T. Jensen, Neuropeptide G protein-coupled receptors as oncotargets, Front. Endocrinol. (Lausanne) 9 (2018) 345, https://doi.org/10.3389/fendo.2018.00345.
[6] C. Morgat, A.K. Mishra, R. Varshney, M. Allard, P. Fernandez, E. Hindié, Targeting neuropeptide receptors for cancer imaging and therapy: perspectives with bombesin, neurotensin, and neuropeptide-Y receptors, J. Nucl. Med. 55 (2014) (2014) 1650–1657, https://doi.org/10.2967/jnumed.114.142000.
[7] A. Abbaci, H. Talbot, S. Saada, N. Gachard, J. Abraham, A. Jaccard, D. Bordessoule, A.L. Fauchais, T. Naves, M.O. Jauberteau, Neurotensin receptor type 2 protects B-cell chronic lymphocytic leukemia cells from apoptosis, Oncogene 37 (2018) 756–767, https://doi.org/10.1038/onc.2017.365.
[8] S. Saada, P. Marget, A.L. Fauchais, M.C. Lise, G. Chemin, P. Sindou, C. Martel, L. Delpy, E. Vidal, A. Jaccard, D. Troutaud, F. Lalloué, M.O. Jauberteau, Differential expression of neurotensin and specific receptors, NTSR1 and NTSR2, in normal and malignant human B lymphocytes, J. Immunol. 189 (2012) 5293–5303, https://doi.org/10.4049/jimmunol.1102937.
[9] S. Béraud-Dufour, C. Devader, F. Massa, M. Roulot, T. Coppola, J. Mazella, Focal adhesion kinase-dependent role of the soluble form of neurotensin receptor-3/sortilin in colorectal cancer cell dissociation, Int. J. Mol. Sci. 17 (2016) E1860, https://doi.org/10.3390/ijms17111860.
[10] T.E. Willnow, C.M. Petersen, A. Nykjaer, VPS10P-domain receptors - regulators of neuronal viability and function, Nat. Rev. Neurosci. 9 (2008) 899–909, https://doi.org/10.1038/nrn2516.
[11] C. Munck Petersen, M.S. Nielsen, C. Jacobsen, J. Tauris, L. Jacobsen, J. Gliemann, S.K. Moestrup, P. Madsen, Propeptide cleavage conditions sortilin/neurotensin receptor-3 for ligand binding, EMBO J. 18 (1999) 595–604, https://doi.org/10.1093/emboj/18.3.595.
[12] M.S. Nielsen, P. Madsen, E.I. Christensen, A. Nykjaer, J. Gliemann, D. Kasper, R. Pohlmann, C.M. Petersen, The sortilin cytoplasmic tail conveys Golgi-endosome transport and binds the VHS domain of the GGA2 sorting protein, EMBO J. 20 (2001) 2180–2190, https://doi.org/10.1093/emboj/20.9.2180.
[13] V. Schmidt, T.E. Willnow, Protein sorting gone wrong–VPS10P domain receptors in cardiovascular and metabolic diseases, Atherosclerosis 245 (2016) 194–199, https://doi.org/10.1016/j.atherosclerosis.2015.11.027.
[14] S. Roselli, J. Pundavela, Y. Demont, S. Faulkner, S. Keene, J. Attia, C.C. Jiang, X.D. Zhang, M.M. Walker, H. Hondermarck, Sortilin is associated with breast cancer aggressiveness and contributes to tumor cell adhesion and invasion, Oncotarget 6 (2015) 10473–10486, https://doi.org/10.18632/oncotarget.3401.
[15] Q. Guo, L. Zhang, F. Li, G. Jiang, The plasticity and potential of leukemia cell lines to differentiate into dendritic cells, Oncol. Lett. 4 (2012) 595–600, https://doi.org/10.3892/ol.2012.821.
[16] E. Huberman, M.F. Callaham, Induction of terminal differentiation in human promyelocytic leukemia cells by tumor-promoting agents, Proc. Natl. Acad. Sci. U. S. A. 76 (1979) 1293–1297, https://doi.org/10.1073/pnas.76.3.1293.
[17] B. Annabi, J.C. Currie, A. Moghrabi, R. Béliveau, Inhibition of HuR and MMP-9 expression in macrophage-differentiated HL-60 myeloid leukemia cells by green tea polyphenol EGCg, Leuk. Res. 31 (2007) 1277–1284, https://doi.org/10.1016/j.leukres.2006.10.001.

[18] A. Vezina, R. Chokor, B. Annabi, EGCG targeting efficacy of NF-κB downstream gene products is dictated by the monocytic/macrophagic differentiation status of promyelocytic leukemia cells, Cancer Immunol. Immunother. 61 (2012) 2321–2331, https://doi.org/10.1007/s00262-012-1301-x.
[19] S.E. Lakhan, A. Kirchgessner, D. Tepper, A. Leonard, Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke, Front. Neurol. 4 (2013) 32, https://doi.org/10.3389/fneur.2013.00032 Erratum in: Front. Neurol. (2018) 9:202.
[20] J. Vandooren, J. Van Damme, G. Opdenakker, On the structure and functions of gelatinase B/matrix metalloproteinase-9 in neuroinflammation, Prog. Brain Res. 214 (2014) 193–206, https://doi.org/10.1016/B978-0-444-63486-3.00009-8.
[21] A. Ben-Baruch, The multifaceted roles of chemokines in malignancy, Cancer Metastasis Rev. 25 (2006) 357–371, https://doi.org/10.1007/s10555-006-9003-5.
[22] T.L. Krasnikova, T.I. Arefieva, E.A. Pylaeva, M.V. Sidorova, Ingramon, a peptide inhibitor of MCP-1 chemokine, reduces migration of blood monocytes stimulated by glioma-conditioned medium, Bull. Exp. Biol. Med. 160 (2016) 480–482, https://doi.org/10.1007/s10517-016-3201-0.
[23] Y. Komohara, K. Ohnishi, J. Kuratsu, M. Takeya, Possible involvement of the M2 anti-inflammatory macrophage phenotype in growth of human gliomas, J. Pathol. 216 (2008) 15–24, https://doi.org/10.1002/path.2370.
[24] G. Seidel, K. Böcker, J. Schulte, C. Wewer, L. Greune, V. Humberg, M.A. Schmidt, Pertussis toxin permeabilization enhances the traversal of Escherichia coli K1, macrophages, and monocytes in a cerebral endothelial barrier model in vitro, Int. J. Med. Microbiol. 301 (2011) 204–212, https://doi.org/10.1016/j.ijmm.2010.08.018.
[25] I. Corraliza, Recruiting specialized macrophages across the borders to restore brain functions, Front. Cell. Neurosci. 8 (2014) 262, https://doi.org/10.3389/fncel.2014.00262.
[26] V.H. Perry, J. Teeling, Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration, Semin. Immunopathol. 35 (2013) 601–612, https://doi.org/10.1007/s00281-013-0382-8.
[27] J. Cheng-Chung Wei, H.C. Huang, W.J. Chen, C.N. Huang, C.H. Peng, C.L. Lin, Epigallocatechin gallate attenuates amyloid β-induced inflammation and neurotoxicity in EOC 13.31 microglia, Eur. J. Pharmacol. 770 (2016) 16–24, https://doi.org/10.1016/j.ejphar.2015.11.048.
[28] Y.J. Lee, D.Y. Choi, Y.P. Yun, S.B. Han, K.W. Oh, J.T. Hong, Epigallocatechin-3-gallate prevents systemic inflammation-induced memory deficiency and amyloidogenesis via its anti-neuroinflammatory properties, J. Nutr. Biochem. 24 (2013) 298–310, https://doi.org/10.1016/j.jnutbio.2012.06.011.
[29] S. Parodi, D.F. Merlo, E. Stagnaro, Working Group for the Epidemiology of Hematolymphopoietic Malignancies in Italy, Coffee and tea consumption and risk of leukaemia in an adult population: a reanalysis of the Italian multicentre case-control study, Cancer Epidemiol. 47 (2017) 81–87, https://doi.org/10.1016/j.canep.2017.01.005.
[30] F.O. Bagger, D. Sasivarevic, S.H. Sohi, L.G. Laursen, S. Pundhir, C.K. Sønderby, O. Winther, N. Rapin, B.T. Porse, BloodSpot: a database of healthy and malignant haematopoiesis updated with purified and single cell mRNA sequencing profiles, Nucleic Acids Res. 47 (2019) D881–D885, https://doi.org/10.1093/nar/gky1076.
[31] R.G. Verhaak, B.J. Wouters, C.A. Erpelinck, S. Abbas, H.B. Beverloo, S. Lugthart, B. Löwenberg, R. Delwel, P.J. Valk, Prediction of molecular subtypes in acute myeloid leukemia based on gene expression profiling, Haematologica 94 (2009) 131–134, https://doi.org/10.3324/haematol.13299.
[32] N. Rapin, F.O. Bagger, J. Jendholm, H. Mora-Jensen, A. Krogh, A. Kohlmann, C. Thiede, N. Borregaard, L. Bullinger, O. Winther, K. Theilgaard-Mönch, B.T. Porse, Comparing cancer vs normal gene expression profiles identifies new disease entities and common transcriptional programs in AML patients, Blood 123 (2014) 894–904, https://doi.org/10.1182/blood-2013-02-485771.
[33] M. Magazin, E. Poszepczynska-Guigné, M. Bagot, L. Boumsell, C. Pruvost, P. Chalon, J.M. Culouscou, P. Ferrara, A. Bensussan, Sezary syndrome cells unlike normal circulating T lymphocytes fail to migrate following engagement of NT1 receptor, J. Invest. Dermatol. 122 (2004) 111–118, https://doi.org/10.1046/j.0022-202X.2003.22131.x.
[34] H.S. Kim, S. Yumkham, J.H. Choi, S.H. Lee, T.H. Kim, S.H. Ryu, P.G. Suh, Neurotensin enhances nitric oxide generation via the JAK2-STAT1 pathway in murine macrophage Raw264.7 cells during costimulation with LPS and IFNgamma, Neuropeptides 40 (2006) 221–229, https://doi.org/10.1016/j.npep.2006.01.001.
[35] L. da Silva, B.M. Neves, L. Moura, M.T. Cruz, E. Carvalho, Neurotensin down-regulates the pro-inflammatory properties of skin dendritic cells and increases epidermal growth factor expression, Biochim. Biophys. Acta 1813 (2011) 1863–1871, https://doi.org/10.1016/j.bbamcr.2011.06.018.
[36] R.J. Turner, F.R. Sharp, Implications of MMP9 for blood brain barrier disruption and hemorrhagic transformation following ischemic stroke, Front. Cell. Neurosci. 10 (2016) 56, https://doi.org/10.3389/fncel.2016.00056.
[37] Y. St-Pierre, C. Van Themsche, P.O. Estève, Emerging features in the regulation of MMP-9 gene expression for the development of novel molecular targets and therapeutic strategies, Curr. Drug Targets Inflamm. Allergy 2 (2003) 206–215, https://doi.org/10.2174/1568010033484133.
[38] A. Asik, C. Kayabasi, B. Ozmen Yelken, S. Yılmaz Susluer, Z.O. Dogan Sigva, T. Balcı Okcanoglu, G. Saydam, C. Biray Avci, C. Gunduz, Antileukemic effect of paclitaxel in combination with metformin in HL-60 cell line, Gene 647 (2018) 213–220, https://doi.org/10.1016/j.gene.2018.01.017.
[39] G.D. Birnie, The HL60 cell line: a model system for studying human myeloid cell differentiation, Br. J. Cancer Suppl. 9 (1988) 41–45 PMCID: PMC2149104.
[40] W.T. Dalton Jr, M.J. Ahearn, K.B. McCredie, E.J. Freireich, S.A. Stass, J.M. Trujillo, HL-60 cell line was derived from a patient with FAB-M2 and not FAB-M3, Blood 71 (1988) 242–247 PMID: 3422031.
[41] S.J. Collins, The HL-60 promyelocytic leukemia cell line: proliferation, differentiation, and cellular oncogene expression, Blood 70 (1987) 1233–1244 PMID: 3311197.
[42] C.M. Wilson, T. Naves, S. Saada, S. Pinet, F. Vincent, F. Lalloué, M.O. Jauberteau, The implications of sortilin/vps10p domain receptors in neurological and human diseases, CNS Neurol. Disord. Drug Targets 13 (2014) 1354–1365, https://doi.org/10.2174/1871527313666141023151642.
[43] R. Chokor, S. Lamy, B. Annabi, Transcriptional targeting of sphingosine-1-phosphate receptor S1P2 by epigallocatechin-3-gallate prevents sphingosine-1-phosphate-mediated signaling in macrophage-differentiated HL-60 promyelomonocytic leukemia cells, Onco Targets Ther. 7 (2014) 667–677, https://doi.org/10.2147/OTT.S62717.
[44] K. Akhtar, S. Ahmad, R.K. Sherwani, Acute promyelocytic leukemia, hypogranular variant: a rare presentation, Clin. Pract. 1 (2011) e11, https://doi.org/10.4081/cp.2011.e11.
[45] S. Béraud-Dufour, T. Coppola, F. Massa, J. Mazella, Neurotensin receptor-2 and -3 are crucial for the anti-apoptotic effect of neurotensin on pancreatic beta-TC3 cells, Int. J. Biochem. Cell Biol. 41 (2009) 2398–2402, https://doi.org/10.1016/j.biocel.2009.04.002.
[46] S. Dupouy, N. Mourra, V.K. Doan, A. Gompel, M. Alifano, P. Forgez, The potential use of the neurotensin high affinity receptor 1 as a biomarker for cancer progression and as a component of personalized medicine in selective cancers, Biochimie 93 (2011) 1369–1378, https://doi.org/10.1016/j.biochi.2011.04.024.
[47] M.B. Mortensen, M. Kjolby, S. Gunnersen, J.V. Larsen, J. Palmfeldt, E. Falk, A. Nykjaer, J.F. Bentzon, Targeting sortilin in immune cells reduces proinflammatory cytokines and atherosclerosis, J. Clin. Invest. 124 (2014) 5317–5322, https://doi.org/10.1172/JCI76002.
[48] B. Annabi, E. Naud, Y.T. Lee, N. Eliopoulos, J. Galipeau, Vascular progenitors derived from murine bone marrow stromal cells are regulated by fibroblast growth factor and are avidly recruited by vascularizing tumors, J. Cell. Biochem. 91 (2004) 1146–1158, https://doi.org/10.1002/jcb.10763.
[49] M. Aghi, K.S. Cohen, R.J. Klein, D.T. Scadden, E.A. Chiocca, Tumor stromal-derived factor-1 recruits vascular progenitors to mitotic neovasculature, where microenvironment influences their differentiated phenotypes, Cancer Res. 66 (2006) 9054–9064, https://doi.org/10.1158/0008-5472.CAN-05-3759.
[50] A. Zgheib, S. Lamy, B. Annabi, Epigallocatechin gallate targeting of membrane type 1 matrix metalloproteinase-mediated Src and Janus kinase/signal transducers and activators of transcription 3 signaling inhibits transcription of colony-stimulating factors 2 and 3 in mesenchymal stromal cells, J. Biol. Chem. 288 (2013) 13378–13386, https://doi.org/10.1074/jbc.M113.456533.
[51] S. Qiu, G. Pellino, F. Fiorentino, S. Rasheed, A. Darzi, P. Tekkis, C. Kontovounisios, A review of the role of neurotensin and its receptors in colorectal cancer, Gastroenterol. Res. Pract. 2017 (2017) 6456257, , https://doi.org/10.1155/2017/6456257.
[52] P. Brun, C. Mastrotto, E. Beggiao, A. Stefani, L. Barzon, G.C. Sturniolo, G. Palù, I. Castagliuolo, Neuropeptide neurotensin stimulates intestinal wound healing following chronic intestinal inflammation, Am. J. Physiol. Gastrointest. Liver Physiol. 288 (2005) G621–G629, https://doi.org/10.1152/ajpgi.00140.2004.
[53] A.M. Piliponsky, C.C. Chen, T. Nishimura, M. Metz, E.J. Rios, P.R. Dobner, E. Wada, K. Wada, S. Zacharias, U.M. Mohanasundaram, J.D. Faix, M. Abrink, G. Pejler, R.G. Pearl, M. Tsai, S.J. Galli, Neurotensin increases mortality and mast cells reduce neurotensin levels in a mouse model of sepsis, Nat. Med. 14 (2008) 392–398, https://doi.org/10.1038/nm1738.
[54] H.W. Koon, Y.S. Kim, H. Xu, A. Kumar, D. Zhao, I. Karagiannides, P.R. Dobner, C. Pothoulakis, Neurotensin induces IL-6 secretion in mouse preadipocytes and adipose tissues during 2,4,6,-trinitrobenzensulphonic acid-induced colitis, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 8766–8771, https://doi.org/10.1073/pnas.0903499106.
[55] D. Zhao, Y. Zhan, H. Zeng, H.W. Koon, M.P. Moyer, C. Pothoulakis, Neurotensin stimulates interleukin-8 expression through modulation of I kappa B alpha phosphorylation and p65 transcriptional activity: involvement of protein kinase C alpha, Mol. Pharmacol. 67 (2005) 2025–2031, https://doi.org/10.1124/mol.104.010801.
[56] R. Goldman, Z. Bar-Shavit, E. Shezen, S. Terry, S. Blumberg, Enhancement of phagocytosis by neurotensin, a newly found biological activity of the neuropeptide, Adv. Exp. Med. Biol. 155 (1982) 133–141, https://doi.org/10.1007/978-1-4684-4394-3_11.
[57] Y. Chen, W. Tan, C. Wang, Tumor-associated macrophage-derived cytokines enhance cancer stem-like characteristics through epithelial-mesenchymal transition, Onco Targets Ther. 11 (2018) 3817–3826, https://doi.org/10.2147/OTT.S168317.
[58] S. Kwon, J.L. Christian, Sortilin associates with transforming growth factor-beta family proteins to enhance lysosome-mediated degradation, J. Biol. Chem. 286 (2011) 21876–21885, https://doi.org/10.1074/jbc.M111.228262.
[59] K. Miyazono, Transforming growth factor-beta signaling in epithelial-mesenchymal transition and progression of cancer, Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 85 (2009) 314–323, https://doi.org/10.2183/pjab.85.314.
[60] K. Miyazono, Y. Katsuno, D. Koinuma, S. Ehata, M. Morikawa, Intracellular and extracellular TGF-β signaling in cancer: some recent topics, Front. Med. 12 (2018) 387–411, https://doi.org/10.1007/s11684-018-0646-8.
[61] H. Ko, Y. So, H. Jeon, M.H. Jeong, H.K. Choi, S.H. Ryu, S.W. Lee, H.G. Yoon, K.C. Choi, TGF-β1-induced epithelial-mesenchymal transition and acetylation of Smad2 and Smad3 are negatively regulated by EGCG in human A549 lung cancer cells, Cancer Lett. 335 (2013) 205–213, https://doi.org/10.1016/j.canlet.2013.02.018.
[62] L.C. Liu, T.C. Tsao, S.R. Hsu, H.C. Wang, T.C. Tsai, J.Y. Kao, T.D. Way, EGCG inhibits transforming growth factor-β-mediated epithelial-to-mesenchymal transition

via the inhibition of Smad2 and Erk1/2 signaling pathways in nonsmall cell lung cancer cells, J. Agric. Food Chem. 60 (2012) 9863–9873, https://doi.org/10.1021/jf303690x.
[63] Y. Wang, N. Liu, X. Su, G. Zhou, G. Sun, F. Du, X. Bian, B. Wang, Epigallocatechin-3-gallate attenuates transforming growth factor-β1 induced epithelial-mesenchymal transition via Nrf2 regulation in renal tubular epithelial cells, Biomed. Pharmacother. 70 (2015) 260–267, https://doi.org/10.1016/j.biopha.2015.01.032.
[64] N. Yang, H. Zhang, X. Cai, Y. Shang, Epigallocatechin-3-gallate inhibits inflammation and epithelial-mesenchymal transition through the PI3K/AKT pathway via upregulation of PTEN in asthma, Int. J. Mol. Med. 41 (2018) 818–828, https://doi.org/10.3892/ijmm.2017.3292.
[65] S.F. Huang, C.T. Horng, Y.S. Hsieh, Y.H. Hsieh, S.C. Chu, P.N. Chen, Epicatechin-3-gallate reverses TGF-β1-induced epithelial-to-mesenchymal transition and inhibits cell invasion and protease activities in human lung cancer cells, Food Chem. Toxicol. 94 (2016) 1–10, https://doi.org/10.1016/j.fct.2016.05.009.
[66] S. Martin, J.P. Vincent, J. Mazella, Involvement of the neurotensin receptor-3 in the neurotensin-induced migration of human microglia, J. Neurosci. 23 (2003) 1198–1205, https://doi.org/10.1523/JNEUROSCI.23-04-01198.2003.
[67] S. Martin, E. Dicou, J.P. Vincent, J. Mazella, Neurotensin and the neurotensin receptor-3 in microglial cells, J. Neurosci. Res. 81 (2005) 322–326, https://doi.org/10.1002/jnr.20477.
[68] Y.Z. Xu, T. Thuraisingam, C. Kanagaratham, S. Tao, D. Radzioch, c-Src kinase is involved in the tyrosine phosphorylation and activity of SLC11A1 in differentiating macrophages, PLoS One 13 (2018) e0196230, , https://doi.org/10.1371/journal.pone.0196230.
[69] J.T. Kim, D.L. Napier, H.L. Weiss, E.Y. Lee, C.M. Townsend Jr, B.M. Evers, Neurotensin receptor 3/sortilin contributes to tumorigenesis of neuroendocrine tumors through augmentation of cell adhesion and migration, Neoplasia 20 (2018) 175–181, https://doi.org/10.1016/j.neo.2017.11.012.
[70] A. Morinville, S. Martin, M. Lavallée, J.P. Vincent, A. Beaudet, J. Mazella, Internalization and trafficking of neurotensin via NTS3 receptors in HT29 cells, Int. J. Biochem. Cell Biol. 36 (2004) 2153–2168, https://doi.org/10.1016/j.biocel.2004.04.013.
[71] S. Martin, V. Navarro, J.P. Vincent, J. Mazella, Neurotensin receptor-1 and -3 complex modulates the cellular signaling of neurotensin in the HT29 cell line, Gastroenterology 123 (2002) 1135–1143, https://doi.org/10.1053/gast.2002.36000.
[72] V. Navarro, J.P. Vincent, J. Mazella, Shedding of the luminal domain of the neurotensin receptor-3/sortilin in the HT29 cell line, Biochem. Biophys. Res. Commun. 298 (2002) 760–764, https://doi.org/10.1016/s0006-291x(02)02564-0.
[73] J. Korbecki, I. Gutowska, I. Kojder, D. Jeżewski, M. Goschorska, A. Łukomska, A. Lubkowska, D. Chlubek, I. Baranowska-Bosiacka, New extracellular factors in glioblastoma multiforme development: neurotensin, growth differentiation factor-15, sphingosine-1-phosphate and cytomegalovirus infection, Oncotarget 9 (2018) 7219–7270, https://doi.org/10.18632/oncotarget.24102.
[74] S.M. Razavi, K.E. Lee, B.E. Jin, P.S. Aujla, S. Gholamin, G. Li, Immune evasion strategies of glioblastoma, Front. Surg. 3 (2016) 11, https://doi.org/10.3389/fsurg.2016.00011.
[75] J. Paupert, V. Mansat-De Mas, C. Demur, B. Salles, C. Muller, Cell-surface MMP-9 regulates the invasive capacity of leukemia blast cells with monocytic features, Cell Cycle 7 (2008) 1047–1053, https://doi.org/10.4161/cc.7.8.5645.